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(54) Title: MATERIALS AND METHODS FOR INCREASING CORN SEED WEIGHT (57) Abstract The subject invention pertains to novel variants of the maize gene, <i>Shrunken2</i> (<i>Sh2</i>) and a method of using that gene. The variant gene, <i>Sh2-m1Rev6</i> , encodes a subunit of the ADP-glucose pyrophosphorylase (AGP) enzyme that has additional amino acids inserted in or near the allosteric binding site of the protein. Corn seed expressing the <i>Sh2-m1Rev6</i> gene has a 15 % weight increase over wild type seed. The increase in seed weight is not associated simply with an increase in percentage starch content of the seed.		

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DESCRIPTIONMATERIALS AND METHODS FOR
INCREASING CORN SEED WEIGHT

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This invention was made with government support under National Science Foundation grant number 93052818. The government has certain rights in this invention.

Cross-Reference to a Related Application

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This application is a continuation-in-part of co-pending application Serial No. 08/299,675, filed September 1, 1994.

Background of the Invention

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ADP-glucose pyrophosphorylase (AGP) catalyzes the conversion of ATP and α -glucose-1-phosphate to ADP-glucose and pyrophosphate. ADP-glucose is used as a glycosyl donor in starch biosynthesis by plants and in glycogen biosynthesis by bacteria. The importance of ADP-glucose pyrophosphorylase as a key enzyme in the regulation of starch biosynthesis was noted in the study of starch deficient mutants of maize (*Zea mays*) endosperm (Tsai and Nelson, 1966; Dickinson and Preiss, 1969). AGP enzymes have been isolated from both bacteria and plants.

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Bacterial AGP consists of a homotetramer, while plant AGP from photosynthetic and non-photosynthetic tissues is a heterotetramer composed of two different subunits. The plant enzyme is encoded by two different genes, with one subunit being larger than the other. This feature has been noted in a number of plants. The AGP subunits in spinach leaf have molecular weights of 54 kDa and 51 kDa, as estimated by SDS-PAGE. Both subunits are immunoreactive with antibody raised against purified AGP from spinach leaves (Copeland and Preiss, 1981; Morell *et al.*, 1987). Immunological analysis using antiserum prepared against the small and large subunits of spinach leaf showed that potato tuber AGP is also encoded by two genes (Okita *et al.*, 1990). The cDNA clones of the two subunits of potato tuber (50 and 51 kDa) have also been isolated and sequenced (Muller-Rober *et al.*, 1990; Nakata *et al.*, 1991).

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As Hannah and Nelson (Hannah and Nelson, 1975 and 1976) postulated, both *Shrunken-2* (*Sh2*) (Bhave *et al.*, 1990) and *Bristle-2* (*Bt2*) (Bae *et al.*, 1990) are structural genes of maize endosperm ADP-glucose pyrophosphorylase. *Sh2* and *Bt2* encode the large subunit and small subunit of the enzyme, respectively. From cDNA sequencing, *Sh2* and *Bt2* proteins have predicted molecular weight of 57,179 Da (Shaw and Hannah, 1992) and 52,224 Da, respectively. The endosperm is the site of most starch deposition during kernel development in maize. *Sh2* and *b12* maize endosperm mutants have greatly reduced starch levels corresponding to deficient levels of AGP activity. Mutations of either gene have been shown to reduce AGP activity by about 95%

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(Tsai and Nelson, 1966; Dickinson and Preiss, 1969). Furthermore, it has been observed that enzymatic activities increase with the dosage of functional wild type *Sh2* and *Bt2* alleles, whereas mutant enzymes have altered kinetic properties. AGP is the rate limiting step in starch biosynthesis in plants. Stark *et al.* placed a mutant form of *E. coli* AGP in potato tuber and obtained a 35% increase in starch content (Stark, 1992).

The cloning and characterization of the genes encoding the AGP enzyme subunits have been reported for various plants. These include *Sh2* cDNA (Bhave *et al.*, 1990), *Sh2* genomic DNA (Shaw and Hannah, 1992), and *Bt2* cDNA (Bae *et al.*, 1990) from maize; small subunit cDNA (Anderson *et al.*, 1989) and genomic DNA (Anderson *et al.*, 1991) from rice; and small and large subunit cDNAs from spinach leaf (Morell *et al.*, 1987) and potato tuber (Muller-Rober *et al.*, 1990; Nakata *et al.*, 1991). In addition, cDNA clones have been isolated from wheat endosperm and leaf tissue (Olive *et al.*, 1989) and *Arabidopsis thaliana* leaf (Lin *et al.*, 1988).

AGP functions as an allosteric enzyme in all tissues and organisms investigated to date. The allosteric properties of AGP were first shown to be important in *E. coli*. A glycogen-overproducing *E. coli* mutant was isolated and the mutation mapped to the structural gene for AGP, designated as *glyC*. The mutant *E. coli*, known as *glyC*-16, was shown to be more sensitive to the activator, fructose 1,6 biphosphate, and less sensitive to the inhibitor, cAMP (Preiss, 1984). Although plant AGP's are also allosteric, they respond to different effector molecules than bacterial AGP's. In plants, 3-phosphoglyceric acid (3-PGA) functions as an activator while phosphate (PO₄) serves as an inhibitor (Dickinson and Preiss, 1969).

In view of the fact that endosperm starch content comprises approximately 70% of the dry weight of the seed, alterations in starch biosynthesis correlate with seed weight. Unfortunately, the undesirable effect associated with such alterations has been an increase in the relative starch content of the seed. Therefore, the development of a method for increasing seed weight in plants without increasing the relative starch content of the seed is an object of the subject invention.

Brief Summary of the Invention

The subject invention concerns a novel variant of the *Shrunken-2* (*Sh2*) gene from maize. The *Sh2* gene encodes ADP-glucose pyrophosphorylase (AGP), an important enzyme involved in starch synthesis in the major part of the corn seed, the endosperm. In a preferred embodiment, the novel gene of the subject invention encodes a variant AGP protein which has two additional amino acids inserted into the sequence. The variant gene described herein has been termed the *Sh2-m1Rev6* gene. Surprisingly, the presence of the *Sh2-m1Rev6* gene in a corn plant results in a substantial increase in corn seed weight when compared to wild type seed weight, but does so in the absence of an increase in the relative starch content of the kernel.

The subject invention further concerns a method of using the variant *sh2* gene in maize to increase seed weight. The subject invention also concerns plants having the variant *sh2* gene and expressing the mutant protein in the seed endosperm.

As described herein, the *sh2* variant, *Sh2-m1Rev6*, can be produced using *in vivo*, site-specific mutagenesis. A transposable element was used to create a series of mutations in the sequence of the gene that encodes the enzyme. As a result, the *Sh2-m1Rev6* gene encodes an additional amino acid pair within or close to the allosteric binding site of the protein.

Brief Description of the Sequences

SEQ ID NO. 1 is the genomic nucleotide sequence of the *Sh2-m1Rev6* gene.
SEQ ID NO. 2 is the nucleotide sequence of the *Sh2-m1Rev6* cDNA.
SEQ ID NO. 3 is the amino acid sequence of the protein encoded by nucleotides 87 through 1640 of SEQ ID NO. 2.
SEQ ID NO. 4 is a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO. 5.
SEQ ID NO. 5 is the amino acid sequence of an ADP-glucose pyrophosphorylase (AGP) enzyme subunit containing a single serine insertion.

Detailed Disclosure of the Invention

The subject invention provides novel variants of the *Shrunken-2* (*Sh2*) gene and a method for increasing seed weight in a plant through the expression of the variant *sh2* gene. The *Sh2* gene encodes a subunit of the enzyme ADP-glucose pyrophosphorylase (AGP) in maize endosperm. One variant gene, denoted herein as *Sh2-m1Rev6*, contains an insertion mutation that encodes an additional tyrosine:serine or serine:tyrosine amino acid pair that is not present in the wild type protein. The sequences of the wild type DNA and protein are disclosed in Shaw and Hannah, 1992. The *in vivo*, site-specific mutation which resulted in the tyrosine:serine or serine:tyrosine insertion, was generated in *Sh2* using the transposable element, *dissociation* (*Ds*), which can insert into, and be excised from, the *Sh2* gene under appropriate conditions. *Ds* excision can alter gene expression through the addition of nucleotides to a gene at the site of excision of the element.

In a preferred embodiment, insertion mutations in the *Sh2* gene were obtained by screening for germinal revertants after excision of the *Ds* transposon from the gene. The revertants were generated by self-pollination of a stock containing the *Ds-Sh2* mutant allele, the *Activator* (*Ac*) element of this transposable element system, and appropriate outside markers. The *Ds* element can transpose when the *Ac* element is present. Wild type seed were selected, planted, self-pollinated and crossed onto a tester stock. Results from this test cross were used to remove wild type alleles due to pollen contamination. Seeds homozygous for each revertant allele were

obtained from the self-progeny. Forty-four germinal revertants of the *Ds*-induced *sh2* mutant were collected.

Cloning and sequencing of the *Ds* insertion site showed that the nucleotide insertion resides in the area of the gene that encodes the binding site for the AGP activator, 3-PGA (Morrell, 1988). Of the 44 germinal revertants obtained, 28 were sequenced. The sequenced revertants defined 5 isoalleles of *sh2*: 13 restored the wild type sequence, 11 resulted in the insertion of the amino acid tyrosine, two contained an additional serine (inserted between amino acid residues 494 and 495, respectively, of the native protein sequence), one revertant contained a two amino acid insertion, tyrosine:tyrosine, and the last one, designated as *Sh2-m1Rev6*, contained the two amino acid insertion, tyrosine:serine or serine:tyrosine. The *Sh2-m1Rev6* variant encodes an AGP enzyme subunit that has either the serine:tyrosine amino acid pair inserted between the glycine and tyrosine at amino acid residues 494 and 495, respectively, of the native protein, or the serine:tyrosine amino acid pair inserted between the two tyrosine residues located at position 495 and 496 of the native protein sequence. Due to the sequence of the amino acids in the area of the insertions, the *Sh2-m1Rev6* variant amino acid sequences encoded by each of these insertions are identical to each other.

Surprisingly, the expression of the *Sh2-m1Rev6* gene in maize resulted in a significant increase in seed weight over that obtained from maize expressing the wild-type *Sh2* allele. Moreover, seeds from plants having the *Sh2-m1Rev6* gene contained approximately the same percentage starch content relative to any of the other revertants generated. In a preferred embodiment, the *Sh2-m1Rev6* gene is contained in homozygous form within the genome of a plant seed.

The subject invention further concerns a plant that has the *Sh2-m1Rev6* gene incorporated into its genome. Other alleles disclosed herein can also be incorporated into a plant genome. In a preferred embodiment, the plant is a monocotyledonous species. More preferably, the plant may be *Zea mays*. Plants having the *Sh2-m1Rev6* gene can be grown from seeds that have the gene in their genome. In addition, techniques for transforming plants with a gene are known in the art.

Because of the degeneracy of the genetic code, a variety of different polynucleotide sequences can encode the variant AGP polypeptide disclosed herein. In addition, it is well within the skill of a person trained in the art to create alternative polynucleotide sequences encoding the same, or essentially the same, polypeptide of the subject invention. These variant or alternative polynucleotide sequences are within the scope of the subject invention. As used herein, references to "essentially the same" sequence refers to sequences which encode amino acid substitutions, deletions, additions, or insertions which do not materially alter the functional activity of the polypeptide encoded by *Sh2-m1Rev6* or the other alleles. The subject invention also contemplates those polynucleotide molecules having sequences which are sufficiently homologous with the wild

type *Sh2* DNA sequence so as to permit hybridization with that sequence under standard high-stringency conditions. Such hybridization conditions are conventional in the art (see, e.g., Maniatis *et al.*, 1989).

The polynucleotide molecules of the subject invention can be used to transform plants to express the *Sh2-m1Rev6* allele, or other alleles of the subject invention, in those plants. In addition, the polynucleotides of the subject invention can be used to express the recombinant variant AGP enzyme. They can also be used as a probe to detect related enzymes. The polynucleotides can also be used as DNA sizing standards.

The polypeptides encoded by the polynucleotides of the subject invention can be used to catalyze the conversion of ATP and α -glucose-1-phosphate to ADP-glucose and pyrophosphate, or to raise an immunogenic response to the AGP enzymes and variants thereof. They can also be used as molecular weight standards, or as an inert protein in an assay.

The following are examples which illustrate procedures and processes, including the best mode, for practicing the invention. These examples should not be construed as limiting, and are not intended to be a delineation of all possible modifications to the technique. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 - Expression of *Sh2-m1Rev6* Gene in Maize Endosperm.

Homozygous plants of each revertant obtained after excision of the *Ds* transposon were crossed onto the F1 hybrid corn, "Florida Stay Sweet." This sweet corn contains a null allele for the *Sh2* gene, termed *sh2-R*. Resulting endosperms contained one dose of the functional allele from a revertant and two female-derived null alleles, denoted by the following genotype *Sh2-m1RevX/sh2-R/sh2-R*, where X represents one of the various isoalleles of the revertants. Crosses were made during two growing seasons.

Resulting seed weight data for each revertant and wild type seed are shown in Table 1. The first column shows the amino acid insertion in the AGP enzyme obtained after the *in vivo*, site-specific mutagenesis.

Table 1.

Sequence alteration	# of revertants	Average Seed weight	Standard deviation
wild type	13	0.250 grams	0.015
tyrosine	11	0.238 grams	0.025
serine	2	0.261 grams	0.014
tyr, tyr	1	0.223 grams	nd*
tyr, ser (Rev6)	1	0.289 grams	0.022

*nd = not determined

The data shown in Table 1 represents the average kernel seed weight for each revertant over the course of two growing seasons. The expression of the *Sh2-m1Rev6* gene to produce the Rev6 mutant AGP subunit gave rise to an almost 16% increase in seed weight in comparison to the wild type revertant. The revertants having the single serine insertion also showed an increase in average seed weight over wild type seed weight.

In addition, starch content was determined on the kernels analyzed above using various methodologies. The analysis showed that *Sh2-m1Rev6* containing kernels were no higher in percentage starch relative to kernels expressing the other alleles shown in the table above. Therefore, it appears that the increase in seed weight is not solely a function of starch content.

Corn seeds that contain at least one functional *Sh2-m1Rev6* allele will be deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852 USA. The culture will be assigned an accession number by the repository.

The subject culture will be deposited under conditions that assure that access to the culture will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 U.S.C. 122. The deposit will be available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposit will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., it will be stored with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the culture. The depositor acknowledges the duty to replace the deposit should the depository be unable to furnish a sample when requested, due to

the condition of the deposit. All restrictions on the availability to the public of the subject culture deposit will be irrevocably removed upon the granting of a patent disclosing it.

5 As would be apparent to a person of ordinary skill in the art, seeds and plants that are homozygous for the *Sh2-m1Rev6* allele can be readily prepared from heterozygous seeds using techniques that are standard in the art. In addition, the *Sh2-m1Rev6* gene can be readily obtained from the deposited seeds.

10 The skilled artisan, using standard techniques known in the art, can also prepare polynucleotide molecules that encode additional amino acid residues, such as serine, at the location of the insertions in the subject revertants. Such polynucleotide molecules are included within the scope of the subject invention.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the scope and purview of this application and the scope of the appended claims.

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SEQUENCE LISTING

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Corn Seed Weight

(iii) NUMBER OF SEQUENCES: 5

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(B) REGISTRATION NUMBER: 38,261
(C) REFERENCE/DOCKET NUMBER: UP146.C1

(ix) TELECOMMUNICATION INFORMATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7745 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCAACTACAC AATGTATTCT TACCTCAGAT GCTTGTCTCTG AAATCTTGT AAGTATCCAC	1860
CTCAATTATT ACTCTTACAT GTTGGTTTAC TTTACGTTG TCTTTTCAAG GGAAATTTAC	1920
TGTATTTTTT GTGTTTGTG GGAGTTCTAT ACTTCTGTTG GACTGGTTAT TGTAAAGATT	1980
TGTTCAAATA GGGTCATCTA ATAATTGTTT GAAATCTGGG AACTGTGGTT TCACTGCGTT	2040

CAGGAAAAAG TGAATTATTG GTTACTGCGT GAATAACTTA TGGAAATAGA CCTTAGAGTT	2100
GCTGCGATGAT TATCACAAAT CATTGCTACG ATATCTTATA ATAGTTCTTT CGACCTCGCA	2160
TTACATATAT AACTGCAACT CCTAGTTGCG TTCAAAAAAA AAAATGCAAC TCTTAGAACG	2220
CTCACCAGTG TAATCTTTCC TGAATTGTTA TTTAATGGCA TGTATGCACT ACTTGTATAC	2280
TTATCTAGGA TTAAGTAATC TAACTCTAGG CCCCATATTT GCAGCATTCT CAAACACAGT	2340
CCTCTAGGAA AAATTATGCT GATGCAAACC GTGTATCTGC TATCATTTTG GCGGAGGCA	2400
CTGGATCTCA GCTCTTTCCT CTGACAAGCA CAAGAGCTAC GCCTGCTGTA AGGGATAACA	2460
CTGAACATCC AACGTTGATT ACTCTATTAT AGTATTATAC AGACTGTACT TTTCGAATTT	2520
ATCTTAGTTT TCTACAATAT TTAGTGGATT CTTCTCATTT TCAAGATACA CAATTGATCC	2580
ATAATCGAAG TGGTATGTAA GACAGTGAGT TAAAAGATTA TATTTTTTGG GAGACTTCCA	2640
GTCAAAATTT CTTAGAAGTT TTTTGGTCC AGATGTTTCA AAAGTCGCG CTTCATACT	2700
TTTTTTAATT TTTTAATTGG TGCACATTA GGTACCTGTT GGAGGATGTT ACAGGCTTAT	2760
TGATATCCCT ATGAGTAACT GCTCAACAG TGGTATAAAT AAGATATTTG TGATGAGTCA	2820
GTTCAATTCT ACTTCGCTTA ACGCCATAT TCATCGTACA TACCTGAAG GCGGGATCAA	2880
CTTTGCTGAT GGATCTGTAC AGGTGATTTA CCTCATCTTG TTGATGTGTA ATACTGTAAT	2940
TAGGAGTAGA TTTGTGTGGA GAGAATAATA AACAGATGCC GAGATTCTTT TCTAAAAGTC	3000
TAGATCCAAA GGCATTGTGG TTCAAACAC TATGGACTTC TACCATTAT GTCATTACTT	3060
TGCCTTAATG TTCCATTGAA TGGGGCAAAT TATTGATTCT ACAAGTGTTT AATTAAAAAC	3120
TAATTGTTCA TCCTGCAGGT ATTAGCGGCT ACACAAATGC CTGAAGAGCC AGCTGGATGG	3180
TTCCAGGGTA CAGCAGACTC TATCAGAAAA TTTATCTGGG TACTCGAGGT AGTTGATATT	3240
TTCTCGTTTA TGAATGTCCA TTTACTCATT CCTGTAGCAT TGTTTCTTTG TAATTTTGAG	3300
TTCTCCTGTA TTTCTTTAGG ATTATTACAG TCACAAATCC ATTGACAACA TTGTAATCTT	3360
GAGTGGCGAT CAGCTTTATC GGATGAATTA CATGGAACCT GTGCAGGTAT GGTGTTCTCT	3420
TGTTCTCAT GTTTCACGTA ATGTCTTGAT TTTGGATTAA CCAACTACTT TTGGCATGCA	3480
TTATTTCCAG AAACATGTCG AGGACGATGC TGATATCACT ATATCATGTG CTCCTGTTGA	3540
TGAGAGGTAA TCAGTTGTTT ATATCATCCT AATATGAATA TGTCATCTTG TTATCCAACA	3600
CAGGATGCAT ATGGTCTAAT CTGCTTTCCT TTTTTTCCC TTCGGAAGCC GAGCTTCTAA	3660
AAATGGGCTA GTGAAGATTG ATCATACTGG ACGTGTAATT CAATTCTTTG AAAAACCCAA	3720
GGGTGCTGAT TTGAATTCTA TGGTTAGAAA TTCCTGTGT AATCCAATTC TTTTGTTTTC	3780
CTTCTTTCT TGAGATGAAC CCTCTTTTA GTTATTCCA TGGATAACCT GTACTTGACT	3840
TATTCAGAAA TGATTTTCTA TTTGCTGTA GAATCTGACA CTAAAGCTAA TAGCACTGAT	3900
GTTGCAGAGA GTTGAGACCA ACTTCCTGAG CTATGCTATA GATGATGCAC AGAAATATCC	3960
ATACCTTGCA TCAATGGGCA TTTATGTCTT CAAGAAAGAT GCACTTTTAG ACCTTCTCAA	4020
GTAATCACTT TCCTGTGACT TATTCTATC CAACTCCTAG TTTACCTTCT AACAGTGTCA	4080

ATTCTTAGGT CAAAATATAC TCAATTACAT GACTTTGGAT CTGAAATCCT CCCAAGAGCT	4140
GTACTAGATC ATAGTGTGCA GGTAAGTCTG ATCTGTCTGG AGTATGTGTT CTGTAAACTG	4200
TAAATTCCTC ATGTCAAAAA GTTGTTTTTG TTTCCAGTTT CCACTACCAA TGCACGATTT	4260
ATGTATTTTC GCTTCCATGC ATCATACATA CTAACAATAC ATTTTACGTA TTGTGTTAGG	4320
CATGCATTTT TACGGGCTAT TGGGAGGATG TTGGAACAAT CAAATCATTC TTTGATGCAA	4380
ACTTGGCCCT CACTGAGCAG GTACTCTGTC ATGTATTCTG TACTGCATAT ATATTACCTG	4440
GAATTC AATG CATAGAATGT GTTAGACCAT CTTAGTTCCA TCCTGTTTTT TTCAATTAGC	4500
TTATCATTTA ATAGTTGTTG GCTAGAATTT AAACACAAAT TTACCTAATA TGTTTCTCTC	4560
TTCAGCCTTC CAAGTTTGAT TTTTACGATC CAAAAACACC TTTCTTCACT GCACCCCGAT	4620
GCTTGCCTCC GACGCAATTG GACAAGTGCA AGGTATATGT CTTACTGAGC ACAATTGTGA	4680
CCTGAGCAAG ATTTTGTGTA CTTGACTTGT TCTCCTCCAC AGATGAAATA TGCATTTATC	4740
TCAGATGGTT GCTTACTGAG AGAATGCAAC ATCGAGCATT CTGTGATTGG AGTCTGCTCA	4800
CGTGTCAGCT CTGGATGTGA ACTCAAGGTA CATACTCTGC CAATGTATCT ACTCTTGAGT	4860
ATACCATTTC AACACCAAGC ATCACC AAT CACACAGAAC AATAGCAACA AAGCCTTTTA	4920
GTTCCAAGCA ATTTAGGGTA GCCTAGAGTT GAAATCTAAC AAAACAAAAG TCAAAGCTCT	4980
ATCACGTGGA TAGTTGTTTT CCATGCACTC TTATTAAAGC TAATTTTTTG GGTATACTAC	5040
ATCCATTTAA TTATTGTTTT ATTGCTTCTT CCCTTTCCTT TCCCCCATT ACTATCGCGT	5100
CTTAAGATCA TACTACGCAC TAGTGTCTTT AGAGGTCTCT GGTGGACATG TTCAAACCAT	5160
CTCAATCGGT GTTGGACAAG TTTTCTTGA ATTTGTGCTA CACCTAACCT ATCAGTATG	5220
TCATCGTTTC AAATCGATC CTTCTGTAT CATCATAAAT CCAATGCAAC ATACGCATTT	5280
ATGCAACATT TATCTGTTGA ACATGTCATC TTTTGTAGG TTAACATTAT GCACCATACA	5340
ATGTAGCATG TCTAATCATC ATCCTATAAA ATTTACATT TAGCTTATGT GGTATCCTCT	5400
TGCCACTTAG AACACCATAT GCTTGATGCC ATTTTCATCCA CCCTGCTTTG ATTCTATGGC	5460
TAACATCTTC ATTAATATCC TCGCCTCTCT GTATCATTGG TCCTAAATAT GGAAATACAT	5520
TCTTCTGCGG CACTACTTGA CCTTCCAAAC TAACGTCTCC TTTGCTCCTT TCTTGTGTGT	5580
AGTAGTACCG AAGTCACATC TCATATATTC GGTTTTAGTT CTACTAAGTC CCGGGTTTGA	5640
TCCCCCTCAG GGGTGAATTT CGGGCTTGGT AAAAAAATC CCCTCGCTGT GTCCCGCCCG	5700
CTCTCGGGGA TCGATATCCT GCGCGCCACC CTCCGGCTGG GCATTGCAGA GTGAGCAGTT	5760
GATCGGCTCG TTAGTGATGG GGAGCGGGGT TCAAGGGTTT TCTCGGCCGG GACCATGTTT	5820
CGGTCTCTTA ATATAATGCC GGGAGGGCAG TCTTCCCTC CCGGTCGAG TTTIAGTTCT	5880
ACCGAGTCTA AAACCTTTGG ACTCTAGAGT CCCCTGTCAC AACTCACAAC TCTAGTTTTT	5940
TATTTACTTC TACCTAGCGT TTATTAATGA TCACTATATC GTCTGTAAAA AGCATACACC	6000
AATGTAATCC CCTTGTATGT CCCTGTGAAT ATTATCCATC ACAAGAAAAA AAGGTAAGGC	6060
TCAAAGTTGA CTTTGTATAT AGTCCTATTC TAATCGAGAA GTCATCTGTA TCTTCGTCTC	6120

TTGTTTGAAC ACTAGTCACA AAATTTTTTG TACATGTTCT TAATGAGTCC AACGTAATAT	6180
TCCTTGATAT TTTGTCATAA GCCCTCATCA AGTCAATGAA AATCACGTGT AGGTCCTTCA	6240
TTTGTTCCCTT ATACTGCTCC ATCACTTGTC TCATTAAGAA AATCTCTCTC ATAGTTAACC	6300
TTTTGGCATG AAACAAAATC ACACAGAAGT TGTTCCTTT TTTAAGATC CCACACAAAA	6360
GAGGTTTGAT CTAAGGAATC TGGATCCCTG ACAGGTTTAT CAAAATCCTT TGTGTTTTTC	6420
TTAAACTGA ATATTCTCC AGCTTCTAGT ATTGATGTAA TATTCAATCT GTTTAGCAAG	6480
TGAACACCTT GGTTCCTGTT GTTACTGTAC CCCCCCCCC CCCCCCCCC CGAGGCCAG	6540
ATTACCACGA CATGAATACA AGAATATTGA ACCCAGATCT AGAGTTTGTT TGTACTGTTG	6600
AAAATCGGTG ACAATTCATT TTGTTATTGC GCTTCTGAT AACGACAGGA CTCCGTGATG	6660
ATGGGAGCGG ACACCTATGA AACTGAAGAA GAAGCTTCAA AGCTACTGTT AGCTGGGAAG	6720
GTCCAGTTG GAATAGGAAG GAACACAAAG ATAAGGTGAG TATGGATGTG GAACACCGG	6780
TTAGTTCCCA AAAATATCAC TCACTGATAC CTGATGGTAT CCTCTGATTA TTTTCAGGAA	6840
CTGTATCATT GACATGAATG CTAGGATTGG GAAGAACGTG GTGATCACAA ACAGTAAGGT	6900
GAGCGAGCGC ACCTACATGG GTGCAGAATC TTGTGTGCTC ATCTATCCTA ATTCGGTAAT	6960
TCCTATCCAG CGCTAGTCTT GTGACCATGG GGCATGGGT CGACTCTGTG ACAGGGCATC	7020
CAAGAGGCTG ATCACCCGGA AGAAGGGTAC TCGTACTACA TAAGGTCTGG AATCGTGGTG	7080
ATCTGAAGA ATGCAACCAT CAACGATGGG TCTGTATAT AGATCGGCTG CGTGTGCGTC	7140
TACAAAACAA GAACCTACAA TGGTATTGCA TCGATGGATC GTGTAACCTT GGTATGGTAA	7200
GAGCCGCTTG ACAGAAAGTC GAGCGTTCGG GCAAGATGCG TAGTCTGGCA TGCTGTTCTT	7260
TGACCATTTG TGCTGCTAGT ATGTACTGTT ATAAGCTGCC CTAGAAGTTG CAGCAAACCT	7320
TTTTATGAAC CTTGTATTT CCATTACCTG CTTTGGATCA ACTATATCTG TCATCCTATA	7380
TATTACTAAA TTTTACGTG TTTTCTAAT TCGGTGCTGC TTTTGGGATC TGGCTTCGAT	7440
GACCGCTCGA CCCTGGGCCA TTGGTTCAGC TCTGTTCTT AGAGCAACTC CAAGGAGTCC	7500
TAAATTTTGT ATTAGATACG AAGGACTTCA GCCGTGTATG TCGTCCTCAC CAAACGCTCT	7560
TTTTGCATAG TGCAGGGGTT GTAGACTTGT AGCCCTTGTT TAAAGAGGAA TTTGAATATC	7620
AAATTATAAG TATTAAATAT ATATTTAATT AGGTTAACAA ATTTGGCTCG TTTTGTCT	7680
TTATTTATGT AATTAGTTTT AAAAATAGAC CTATATTTC ATACGAAATA TCATTAACAT	7740
CGATA	7745

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1919 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACAAGATCAC TTCGGGAGGC AAGTGCGATT TTGATCTTGC AGCCACCTTT TTTGTTCTG	60
TTGTGTATCT AGTAGTTGGA GGAGATATGC AGTTTGCAC TGCATTGGAC ACGAACTCAG	120
GTCCTCACCA GATAAGATCT TGTGAGGGTG ATGGGATTGA CAGGTTGGAA AAATTAAGTA	180
TTGGGGGCGAG AAAGCAGGAG AAAGCTTTGA GAAATAGGTG CTTTGGTGGT AGAGTTGCTG	240
CAACTACACA ATGTATTCTT ACCTCAGATG CTGTCTCTGA AACTCTTCAT TCTCAAACAC	300
AGTCTCTAG GAAAAATTAT GCTGATGCAA ACCGTGTATC TGCATCATT TTGGGCGGAG	360
GCACTGGATC TCAGCTCTTT CCTCTGACAA GCACAAGAGC TACGCCCTGCT GTACCTGTTG	420
GAGGATGTTA CAGGCTTATT GATATCCCTA TGAGTAACTG CTTCAACAGT GGTATAAATA	480
AGATATTTGT GATGAGTCAG TTCAATTCTA CTTGCTTAA CCGCCATATT CATCGTACAT	540
ACCTTGAAGG CGGGATCAAC TTTGCTGATG GATCTGTACA GGTATTAGCG GCTACACAAA	600
TGCCTGAAGA GCCAGCTGGA TGGTTCCAGG GTACAGCAGA CTCTATCAGA AAATTATCT	660
GGGTACTCGA GGATTATTAC AGTCACAAAT CCATTGACAA CATTGTAATC TTGAGTGGCG	720
ATCAGCTTTA TCGGATGAAT TACATGGAAC TTGTGCAGAA ACATGTCGAG GACGATGCTG	780
ATATCACTAT ATCATGTGCT CCTGTTGATG AGAGCCGAGC TTCTAAAAAT GGGCTAGTGA	840
AGATTGATCA TACTGGACGT GTACTTCAAT TCTTTGAAAA ACCAAAGGGT GCTGATTGTA	900
ATTCTATGAG AGTTGAGACC AACTTCCTGA GCTATGCTAT AGATGATGCA CAGAAATATC	960
CATACCTTGC ATCAATGGGC ATTTATGTCT TCAAGAAAGA TGCACTTTTA GACCTTCTCA	1020
AGTCAAATA TACTCAATTA CATGACTTTG GATCTGAAAT CCTCCAAGA GCTGTACTAG	1080
ATCATAGTGT GCAGGCATGC ATTTTACGG GCTATTGGGA GGATGTTGGA ACAATCAAAT	1140
CATTCTTTGA TGCAAACTG GCCCTCACTG AGCAGCCTTC CAAGTTTGAT TTTTACGATC	1200
CAAAAACACC TTTCTTCACT GCACCCCGAT GCTTGCCCTC GACGCAATTG GACAAGTGCA	1260
AGATGAAATA TGCAATTTATC TCAGATGGTT GCTTACTGAG AGAATGCAAC ATCGAGCATT	1320
CTGTGATTGG AGTCTGCTCA CGTGTCAGCT CTGGATGTGA ACTCAAGGAC TCCGTGATGA	1380
TGGGAGCGGA CATCTATGAA ACTGAAGAAG AAGCTTCAAA GCTACTGTTA GCTGGGAAGG	1440
TCCCGATTGG AATAGGAAGG AACACAAAGA TAAGGAACTG TATCATTGAC ATGAATGCTA	1500
GGATTGGGAA GAACGTGGTG ATCACAACA GTAAGGGCAT CCAAGAGGCT GATCACCCGG	1560
AAGAAGGGTA CTCGTACTAC ATAAGGTCTG GAATCGTGGT GATCCTGAAG AATGCAACCA	1620
TCAACGATGG GTCTGTCATA TAGATCGGCT GCGTTTGGCT CTACAAACA AGAACCTACA	1680
ATGGTATTGC ATCGATGGAT CGTGTAACCT TGGTATGGTA AGAGCCGCTT GACAGGAAGT	1740
CGAGCTTCGG GCGAAGATGC TAGTCTGGCA TGCTGTTCTT TGACCATTG TGCTGCTAGT	1800
ATGTACCTGT TATAAGCTGC CCTAGAAGTT GCAGCAAACC TTTTATGAA CCTTTGTATT	1860
TCCATTACCC TGCTTTGGAT CAACTATATC TGTCAGTCCT ATATATTACT AAATTTTAA	1919

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 518 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Gln Phe Ala Leu Ala Leu Asp Thr Asn Ser Gly Pro His Gln Ile
 1           5           10           15
Arg Ser Cys Glu Gly Asp Gly Ile Asp Arg Leu Glu Lys Leu Ser Ile
          20           25           30
Gly Gly Arg Lys Gln Glu Lys Ala Leu Arg Asn Arg Cys Phe Gly Gly
          35           40           45
Arg Val Ala Ala Thr Thr Gln Cys Ile Leu Thr Ser Asp Ala Cys Pro
          50           55           60
Glu Thr Leu His Ser Gln Thr Gln Ser Ser Arg Lys Asn Tyr Ala Asp
65           70           75           80
Ala Asn Arg Val Ser Ala Ile Ile Leu Gly Gly Gly Thr Gly Ser Gln
          85           90           95
Leu Phe Pro Leu Thr Ser Thr Arg Ala Thr Pro Ala Val Pro Val Gly
          100          105          110
Gly Cys Tyr Arg Leu Ile Asp Ile Pro Met Ser Asn Cys Phe Asn Ser
          115          120          125
Gly Ile Asn Lys Ile Phe Val Met Ser Gln Phe Asn Ser Thr Ser Leu
          130          135          140
Asn Arg His Ile His Arg Thr Tyr Leu Glu Gly Gly Ile Asn Phe Ala
145          150          155          160
Asp Gly Ser Val Gln Val Leu Ala Ala Thr Gln Met Pro Glu Glu Pro
          165          170          175
Ala Gly Trp Phe Gln Gly Thr Ala Asp Ser Ile Arg Lys Phe Ile Trp
          180          185          190
Val Leu Glu Asp Tyr Tyr Ser His Lys Ser Ile Asp Asn Ile Val Ile
          195          200          205
Leu Ser Gly Asp Gln Leu Tyr Arg Met Asn Tyr Met Glu Leu Val Gln
          210          215          220
Lys His Val Glu Asp Asp Ala Asp Ile Thr Ile Ser Cys Ala Pro Val
225          230          235          240
Asp Glu Ser Arg Ala Ser Lys Asn Gly Leu Val Lys Ile Asp His Thr
          245          250          255
Gly Arg Val Leu Gln Phe Phe Glu Lys Pro Lys Gly Ala Asp Leu Asn
          260          265          270
Ser Met Arg Val Glu Thr Asn Phe Leu Ser Tyr Ala Ile Asp Asp Ala
          275          280          285

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Gln Lys Tyr Pro Tyr Leu Ala Ser Met Gly Ile Tyr Val Phe Lys Lys
 290 295 300
 Asp Ala Leu Leu Asp Leu Leu Lys Ser Lys Tyr Thr Gln Leu His Asp
 305 310 315 320
 Phe Gly Ser Glu Ile Leu Pro Arg Ala Val Leu Asp His Ser Val Gln
 325 330 335
 Ala Cys Ile Phe Thr Gly Tyr Trp Glu Asp Val Gly Thr Ile Lys Ser
 340 345 350
 Phe Phe Asp Ala Asn Leu Ala Leu Thr Glu Gln Pro Ser Lys Phe Asp
 355 360 365
 Phe Tyr Asp Pro Lys Thr Pro Phe Phe Thr Ala Pro Arg Cys Leu Pro
 370 375 380
 Pro Thr Gln Leu Asp Lys Cys Lys Met Lys Tyr Ala Phe Ile Ser Asp
 385 390 395 400
 Gly Cys Leu Leu Arg Glu Cys Asn Ile Glu His Ser Val Ile Gly Val
 405 410 415
 Cys Ser Arg Val Ser Ser Gly Cys Glu Leu Lys Asp Ser Val Met Met
 420 425 430
 Gly Ala Asp Ile Tyr Glu Thr Glu Glu Glu Ala Ser Lys Leu Leu Leu
 435 440 445
 Ala Gly Lys Val Pro Ile Gly Ile Gly Arg Asn Thr Lys Ile Arg Asn
 450 455 460
 Cys Ile Ile Asp Met Asn Ala Arg Ile Gly Lys Asn Val Val Ile Thr
 465 470 475 480
 Asn Ser Lys Gly Ile Gln Glu Ala Asp His Pro Glu Glu Gly Tyr Ser
 485 490 495
 Tyr Tyr Ile Arg Ser Gly Ile Val Val Ile Leu Lys Asn Ala Thr Ile
 500 505 510
 Asn Asp Gly Ser Val Ile
 515

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1551 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATGCAGTTTG CACTTGCATT GGACACGAAC TCAGGTCCTC ACCAGATAAG ATCTTGTGAG	60
GGTGATGGGA TTGACAGCTT GGAATAATTA AGTATTGGGG GCAGAAAGCA GGAGAAAGCT	120
TTGAGAAATA GGTGCTTTGG TGGTAGAGTT GCTGCAACTA CACAATGTAT TCTTACCTCA	180
GATGCTTGTC CTGAAACTCT TCATTCTCAA ACACAGTCCT CTAGGAAAAA TTATGCTGAT	240
GCAAACCGTG TATCTGCGAT CATTTTGGGC GGAGGCACTG GATCTCAGCT CTTTCCTCTG	300

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ACAAGCACAA GAGCTACGCC TGCTGTACCT GTTGGAGGAT GTTACAGGCT TATTGATATC 360
CCTATGAGTA ACTGCTTCAA CAGTGGTATA AATAAGATAT TTGTGATGAG TCAGTTCAAT 420
TCTACTTCGC TTAACCGCCA TATTCATCGT ACATACCTTG AAGGCGGGAT CAACCTTGCT 480
GATGGATCTG TACAGGTATT AGCGGCTACA CAAATGCCTG AAGAGCCAGC TGGATGGTTC 540
CAGGGTACAG CAGACTCTAT CAGAAAATTT ATCTGGGTAC TCGAGGATTA TTACAGTCAC 600
AAATCCATTG ACAACATTGT AATCTTGAGT GGCGATCAGC TTTATCGGAT GAATTACATG 660
GAACCTGTGC AGAAACATGT CGAGGACGAT GCTGATATCA CTATATCATG TGCTCCTGTT 720
GATGAGAGCC GAGCTTCTAA AAATGGGCTA GTGAAGATTG ATCATACTGG ACGTGTACTT 780
CAATTCTTTG AAAAACCAAA GGGTGCTGAT TTGAATTCTA TGAGAGTTGA GACCAACTTC 840
CTGAGCTATG CTATAGATGA TGCACAGAAA TATCCATACC TTGCATCAAT GGGCATTAT 900
GTCTTCAAGA AAGATGCACT TTTAGACCTT CTCAAGTCAA AATATACTCA ATTACATGAC 960
TTTGGATCTG AAATCCTCCC AAGAGCTGTA CTAGATCATA GTGTGCAGGC ATGCATTTTT 1020
ACGGGCTATT GGGAGGATGT TGGAACAATC AAATCATTCT TTGATGCAAA CTTGGCCCTC 1080
ACTGAGCAGC CTTCCAAGTT TGATTTTTAC GATCCAAAAA CACCTTTCTT CACTGCACCC 1140
CGATGCTTGC CTCCGACGCA ATTGGACAAG TGCAAGATGA AATATGCATT TATCTCAGAT 1200
GGTTGCTTAC TGAGAGAATG CAACATCGAG CATTCTGTGA TTGGAGTCTG CTCACGTGTC 1260
AGCTCTGGAT GTGAACTCAA GGACTCCGTG ATGATGGGAG CGGACATCTA TGAAACTGAA 1320
GAAGAAGCTT CAAAGCTACT GTTAGCTGGG AAGGTCCCGA TTGGAATAGG AAGGAACACA 1380
AAGATAAGGA ACTGTATCAT TGACATGAAT GCTAGGATTG GGAAGACGT GGTGATCACA 1440
AACAGTAAGG GCATCCAAGA GGCTGATCAC CCGGAAGAAG GGTCTACTA CATAAGGTCT 1500
GGAATCGTGG TGATCCTGAA GAATGCAACC ATCAACGATG GGTCTGTCAT A 1551

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(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 517 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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Met Gln Phe Ala Leu Ala Leu Asp Thr Asn Ser Gly Pro His Gln Ile
1           5           10           15
Arg Ser Cys Glu Gly Asp Gly Ile Asp Arg Leu Glu Lys Leu Ser Ile
20           25           30
Gly Gly Arg Lys Gln Glu Lys Ala Leu Arg Asn Arg Cys Phe Gly Gly
35           40           45
Arg Val Ala Ala Thr Thr Gln Cys Ile Leu Thr Ser Asp Ala Cys Pro
50           55           60

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Glu Thr Leu His Ser Gln Thr Gln Ser Ser Arg Lys Asn Tyr Ala Asp
 65 70 75 80
 Ala Asn Arg Val Ser Ala Ile Il Leu Gly Gly Gly Thr Gly Ser Gln
 85 90 95
 Leu Phe Pro Leu Thr Ser Thr Arg Ala Thr Pro Ala Val Pro Val Gly
 100 105 110
 Gly Cys Tyr Arg Leu Ile Asp Ile Pro Met Ser Asn Cys Phe Asn Ser
 115 120 125
 Gly Ile Asn Lys Ile Phe Val Met Ser Gln Phe Asn Ser Thr Ser Leu
 130 135 140
 Asn Arg His Ile His Arg Thr Tyr Leu Glu Gly Gly Ile Asn Phe Ala
 145 150 155 160
 Asp Gly Ser Val Gln Val Leu Ala Ala Thr Gln Met Pro Glu Glu Pro
 165 170 175
 Ala Gly Trp Phe Gln Gly Thr Ala Asp Ser Ile Arg Lys Phe Ile Trp
 180 185 190
 Val Leu Glu Asp Tyr Tyr Ser His Lys Ser Ile Asp Asn Ile Val Ile
 195 200 205
 Leu Ser Gly Asp Gln Leu Tyr Arg Met Asn Tyr Met Glu Leu Val Gln
 210 215 220
 Lys His Val Glu Asp Asp Ala Asp Ile Thr Ile Ser Cys Ala Pro Val
 225 230 235 240
 Asp Glu Ser Arg Ala Ser Lys Asn Gly Leu Val Lys Ile Asp His Thr
 245 250 255
 Gly Arg Val Leu Gln Phe Phe Glu Lys Pro Lys Gly Ala Asp Leu Asn
 260 265 270
 Ser Met Arg Val Glu Thr Asn Phe Leu Ser Tyr Ala Ile Asp Asp Ala
 275 280 285
 Gln Lys Tyr Pro Tyr Leu Ala Ser Met Gly Ile Tyr Val Phe Lys Lys
 290 295 300
 Asp Ala Leu Leu Asp Leu Leu Lys Ser Lys Tyr Thr Gln Leu His Asp
 305 310 315 320
 Phe Gly Ser Glu Ile Leu Pro Arg Ala Val Leu Asp His Ser Val Gln
 325 330 335
 Ala Cys Ile Phe Thr Gly Tyr Trp Glu Asp Val Gly Thr Ile Lys Ser
 340 345 350
 Phe Phe Asp Ala Asn Leu Ala Leu Thr Glu Gln Pro Ser Lys Phe Asp
 355 360 365
 Phe Tyr Asp Pro Lys Thr Pro Phe Phe Thr Ala Pro Arg Cys Leu Pro
 370 375 380
 Pro Thr Gln Leu Asp Lys Cys Lys Met Lys Tyr Ala Phe Ile Ser Asp
 385 390 395 400
 Gly Cys Leu Leu Arg Glu Cys Asn Ile Glu His Ser Val Ile Gly Val
 405 410 415

Cys Ser Arg Val Ser Ser Gly Cys Glu Leu Lys Asp Ser Val Met Met
 420 425 430
 Gly Ala Asp Ile Tyr Glu Thr Glu Glu Glu Ala Ser Lys Leu Leu Leu
 435 440 445
 Ala Gly Lys Val Pro Ile Gly Ile Gly Arg Asn Thr Lys Ile Arg Asn
 450 455 460
 Cys Ile Ile Asp Met Asn Ala Arg Ile Gly Lys Asn Val Val Ile Thr
 465 470 475 480
 Asn Ser Lys Gly Ile Gln Glu Ala Asp His Pro Glu Glu Gly Ser Tyr
 485 490 495
 Tyr Ile Arg Ser Gly Ile Val Val Ile Leu Lys Asn Ala Thr Ile Asn
 500 505 510
 Asp Gly Ser Val Ile
 515

Claims

- 1 1. A polynucleotide molecule, comprising a variant of the wild type *shrunkn-2* (*Sh2*)
2 gene, wherein said variant codes for the insertion of at least one additional amino acid within or
3 close to the allosteric binding site of the ADP-glucose pyrophosphorylase (AGP) enzyme subunit,
4 whereby a plant expressing said polynucleotide molecule has increased seed weight relative to the
5 seed weight of a plant expressing the wild type *Sh2* gene.
- 1 2. The polynucleotide molecule, according to claim 1, wherein said polynucleotide
2 molecule encodes at least one serine residue inserted between amino acids 494 and 495 of the
3 native AGP enzyme subunit.
- 1 3. The polynucleotide molecule, according to claim 1, wherein said polynucleotide
2 molecule encodes the amino acid pair tyrosine:serine, wherein said amino acid pair is inserted
3 between amino acids 494 and 495 of the native AGP enzyme subunit.
- 1 4. The polynucleotide molecule, according to claim 1, wherein said polynucleotide
2 molecule encodes the amino acid pair serine:tyrosine, wherein said amino acid pair is inserted
3 between amino acids 495 and 496 of the native AGP enzyme subunit.
- 1 5. The polynucleotide molecule, according to claim 1, wherein the AGP enzyme encoded
2 by said polynucleotide molecule consists essentially of an amino acid sequence selected from the
3 group consisting of SEQ ID NO. 5 and SEQ ID NO. 3.
- 1 6. The polynucleotide molecule, according to claim 5, wherein the nucleotide sequence
2 encoding SEQ ID NO. 3 comprises nucleotides 87 through 1640 of the sequence shown in SEQ
3 ID NO. 2 or a degenerate fragment thereof.
- 1 7. A method for increasing the seed weight of a plant, comprising incorporating the
2 polynucleotide molecule of claim 1 into the genome of said plant and expressing the protein
3 encoded by said polynucleotide molecule.
- 1 8. The method, according to claim 7, wherein said plant is *Zea mays*.
- 1 9. A plant seed comprising the polynucleotide molecule of claim 1 within the genome
2 of said seed.

- 1 10. A plant expressing the polynucleotide molecule of claim 1.
- 2 11. The plant, according to claim 10, wherein said plant is *Zea mays*.
- 1 12. The plant, according to claim 10, wherein said plant is grown from the seed of claim
2 9.
- 1 13. A variant ADP-glucose pyrophosphorylase (AGP) protein, wherein said protein has
2 at least one additional amino acid inserted within or close to the allosteric binding site of the
3 wild-type AGP protein.
- 1 14. The variant AGP protein, according to claim 13, wherein said protein has at least one
2 serine residue inserted between amino acids 494 and 495 of the wild type AGP protein sequence.
- 1 15. The variant AGP protein, according to claim 11, wherein said protein has the amino
2 acid pair tyrosine:serine inserted between amino acids 494 and 495 of the wild-type AGP protein
3 sequence.
- 1 16. The variant AGP protein, according to claim 11, wherein said protein has the amino
2 acid pair serine:tyrosine inserted between amino acids 495 and 496 of the wild-type AGP protein
3 sequence.
- 1 17. The variant AGP protein, according to claim 13, wherein said protein consists
2 essentially of an amino acid sequence selected from the group consisting of SEQ ID NO. 5 and
3 SEQ ID NO. 3.
- 1 18. The variant AGP protein, according to claim 13, wherein said protein is expressed
2 in the endosperm of a plant during seed development.



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(54) Title: MATERIALS AND METHODS FOR INCREASING CORN SEED WEIGHT (57) Abstract The subject invention pertains to novel variants of the maize gene, <i>Shrunken2</i> (<i>Sh2</i>) and a method of using that gene. The variant gene, <i>Sh2-m1Rev6</i> , encodes a subunit of the ADP-glucose pyrophosphorylase (AGP) enzyme that has additional amino acids inserted in or near the allosteric binding site of the protein. Corn seed expressing the <i>Sh2-m1Rev6</i> gene has a 15 % weight increase over wild type seed. The increase in seed weight is not associated simply with an increase in percentage starch content of the seed.		

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N9/12 C12N15/54 A01H5/00 A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M.J. GIROUX ET AL.: "A single gene mutation that increases maize seed weight" PROC. NATL. ACAD. SCI. USA, vol. 95, no. 12, 11 June 1996, pages 5824-9, XP000652281 see the whole document.	1-18
A	M.R. BHAVE ET AL.: "Identification and molecular characterization of Shrunken-2 cDNA clones of maize" PLANT CELL, vol. 2, 1990, pages 581-8, XP000652283 cited in the application see the abstract.	1

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